REMARKS

The foregoing amendments are made to insert the sequence identification numbers into the specification. No new matter has been added.

Respectfully submitted,

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Version with markings to show changes made

In the specification:

Paragraph beginning on page 18, line 20 has been amended as follows:
Polypeptide binding molecules of the invention advantageously contain protein-binding motifs, such as protein dimerization motifs as known in the art. Examples of a protein-binding motifs include the tetratricopeptide repeat (TPR) which is found in proteins associated with multiprotein complexes (Blatch and Lassle, 1999, Bioessays 21, 932-9), the Arg-Gly-Asp-Ser found in multimerin (Hayward 1997, Clin Invest Med 20, 176-87), the LXCXE (SEQ ID NO:60) motif found in SV40 Large T antigen necessary for binding to p53 protein (DeCaprio 1999, Biologicals 27, 23-8), the C-terminal VXI motif of ABP, which mediates binding of ABP to GluR2/3 through a Class I PDZ interaction to form homodimers and heteromultimers (Srivastava and Ziff, Ann N Y Acad Sci 868, 561-4), as well as the conserved Ran-binding motif found in species from yeasts to mammals (Seki et al, 1996, J Biochem (Tokyo), 120, 207-14).

Paragraph beginning on page 26, line 4 has been amended as follows:
In general, a preferred zinc finger framework has the structure:

(A) X_{0-2} **C** X_{1-5} **C** X_{9-14} **H** X_{3-6} $^{\mathbf{H}}/_{\mathbf{C}}$ (SEQ ID NO:4)

where X is any amino acid, and the numbers in subscript indicate the possible numbers of residues represented by X.

Paragraph beginning on page 26, line 11 has been amended as follows:
In a preferred aspect of the present invention, zinc finger nucleic acid binding motifs may be represented as motifs having the following primary structure:

(B) X^a **C** X_{2-4} **C** X_{2-3} F X^c X X X X L X X **H** X X X^b **H** - linker (SEQ ID NO:5)
-1 1 2 3 4 5 6 7 8 9

wherein X (including X^a, X^b and X^c) is any amino acid. X_{2-4} and X_{2-3} refer to the presence of 2 or 4, or 2 or 3, amino acids, respectively. The Cys and His residues, which together co-ordinate the zinc metal atom, are marked in bold text and are usually invariant, as is the Leu residue at position +4 in the α-helix.

Paragraph beginning on page 27, line 16 has been amended as follows:
Preferably, the linker is T-G-E-K (SEQ ID NO:6) or T-G-E-K-P (SEQ ID NO:7).

Paragraph beginning on page 29, line 7 has been amended as follows:

Consensus zinc finger structures may be prepared by comparing the sequences of known zinc fingers, irrespective of whether their binding domain is known. Preferably, the consensus structure is selected from the group consisting of the consensus structure P Y K C P E C G K S F S Q K S D L V K H Q R T H T G (SEQ ID NO:8), and the consensus structure P Y K C S E C G K A F S Q K S N L T R H Q R I H T G E K P (SEQ ID NO:9).

Paragraph beginning on page 29, line 13 has been amended as follows:
The consensuses are derived from the consensus provided by Krizek *et al.*, (1991) J. Am. Chem. Soc. 113: 4518-4523 and from Jacobs, (1993) PhD thesis, University of Cambridge, UK. In both cases, the linker sequences described above for joining two zinc finger motifs together, namely TGEK (SEQ ID NO:6) or TGEKP (SEQ ID NO:7) can be formed on the ends of the consensus. Thus, a P may be removed where necessary, or, in the case of the consensus terminating T G, E K (P) can be added.

Paragraph beginning on page 31, line 30 has been amended as follows:
A "leader" peptide may be added to the N-terminal finger. Preferably, the leader peptide is MAEEKP (SEQ ID NO:10).

Paragraph beginning on page 32, line 28 has been amended as follows:
The DNA binding site for the TFIIIAZif protein contains the DNA recognition sites for zinc fingers 1-3 of TFIIIA and the three zinc fingers of Zif 268. These are the DNA sequences GGATGGGAGAC (SEQ ID NO:61) and GCGTGGGCGT (SEQ ID NO:62), respectively, as shown in Sequence ID 3. The six base pair sequence GTACCT in Sequence ID 3 is a spacer region of DNA that separates the two binding sites and the nucleotide composition of the DNA spacer appears to have no effect on binding of the protein. Therefore, this or other structured linkers could be used with other DNA spacers of different length and sequence.

Paragraph beginning on page 33, line 4 has been amended as follows:
The amino Acid Sequence of Zinc Finger 4 of TFIIIA, including the flanking sequences as used in the composite protein of the invention, is
NIKICVYVCHFENCGKAFKKHNQLK VHQFSHTQQLP (SEQ ID NO:63).

Paragraph beginning on page 33, line 8 has been amended as follows:
The nucleotide Sequence of Zinc Finger 4 of TFIIIA, including the flanking sequences, is
AACATCAAGATCTGCGTCTATGTGTGCCATTTTGAGAACTGTGGCAAAGCATT
CAAGAAACACAATCAATTAAAGGTTCATCAGTTCAGTCACACACAGCAGCTG
CCG (SEQ ID NO:64).

Paragraph beginning on page 47, line 20 has been amended as follows:

Plants also have a preference for certain nucleotides adjacent to the ATG encoding the initiating methionine and for most efficient translation, these nucleotides may be modified. To facilitate translation in plant cells, it is preferable to insert, immediately upstream of the ATG representing the initiating methionine of the gene to be expressed, a "plant translational initiation context sequence". A variety of sequences can be inserted at this position. These include the sequence the sequence 5'-AAGGAGATATAACAATG-3' (SEQ ID NO:11) (Prasher *et al.* Gene 111: 229-233 (1992); Chalfie *et al.* Science 263: 802-805 (1992)), the sequence 5'-GTCGACCATG-3' (SEQ ID NO:12) (Clontech 1993/1994 catalog, page 210), and the sequence 5'-TAAACAATG-3' (Joshi *et al.* Nucl. Acids Res. 15: 6643-6653 (1987)). For any particular plant species, a survey of natural sequences available in any databank (*e.g.* GenBank) can be undertaken to determine preferred "plant translational initiation context sequences" on a species-by-species basis.

Paragraph beginning on page 85, line 1 has been amended as follows:
5' CTCCTGCAGTTGGACCTGTGCCATGGCCGGCTGGGCCGCATAGAATGG AACAACTAAAGC 3' (SEQ ID NO:13) [(Seq ID No. 1)]

which anneals in the region of the polylinker. Electrocompetent DH5α cells are transformed with recombinant vector in 200ng aliquots, grown for 1 hour in 2xTY medium with 1% glucose, and plated on TYE containing 15 µg/ml tetracycline and 1% glucose.

Paragraph beginning on page 85, line 9 has been amended as follows:
The zinc finger phage display library of the present invention contains amino acid randomisations in putative base-contacting positions from the second and third zinc fingers of the three-finger DNA binding domain of Zif268, and contains members that bind DNA of the sequence XXXXXGGCG where X is any base. Further details of the library used may be found in WO 98/53057, which is incorporated herein by reference. The DNA sequences AAAAAAGGCG (SEQ ID NO:14) and AAAAAAGGCGAAAAAA (SEQ ID NO:15) are used as selection targets in this example because short runs of adenines can cause intrinsic DNA bending - moreover, the structure of the bend can be disrupted by binding of the antibiotic distamycin A.

Paragraph beginning on page 86, line 30 has been amended as follows:

	F1	SEQ	F2	SEQ	F3	SEQ
	-1123456	ID NO	-1123456	ID NO	-1123456	ID NO
Clone 1	RSDELTR	16	RSDDLST	17	TNNTRIK	20

Clone 2	RSDELTR	16	RSDDLST	17	HKATRIK	21
Clone 3	RSDELTR	16	RSDDLST	17	TDKVRKK	22
Clone 4	RSDELTR	16	RSDDLST	17	HNASRIN	23
Clone 5	RSDELTR	16	RSDDLSV	18	TNNSRKK	24
Clone 6	RSDELTR	16	RSDDLST	17	TNATRKK	25
Clone 7	RSDELTR	16	RSDDLSQ	19	TRNTRKN	26
Clone 8	RSDELTR	16	RSDDLSV	18	TNNSRKN	27

Paragraph beginning on page 87, line 19 has been amended as follows:
Clones 1-4 were selected to bind the oligo:
tataAAAAAAGGCGTGtcacagtcagtccacacgtc (SEQ ID NO:28)

Paragraph beginning on page 87, line 22 has been amended as follows:
Clones 5-8 were selected to bind the oligo:
tataAAAAAAGGCGAAAAAAtcacagtcagtccacacgtc (SEQ ID NO:29)

Paragraph beginning on page 88, line 26 has been amended as follows:
The amino acid sequences from the helical regions of the selected zinc fingers were sequenced as:

clone 1 RSDELTRHIRIH (SEQ ID NO:30) RSDTLSVHIRTH (SEQ ID NO:31) HNAHRKTHTKIH (SEQ ID NO:32)
clone 6 RSDELTRHIRIH (SEQ ID NO:30) RSDHLSVHIRTH (SEQ ID NO:33) KKFAHSAHRKTHTKIH (SEQ ID NO:34)

These two clones were selected using the oligo:
tatacaAGCTTGGCGatcacagtcagtccacacgtc (SEQ ID NO:35)

Paragraph beginning on page 89, line 31 has been amended as follows:
Four different clones were selected using the DNA library tatagtYRYRYGGCG atcacagtcagtccacacgtc (SEQ ID NO:36) in the presence of echinomycin (see Figure 3).

Paragraph beginning on page 90, line 2 has been amended as follows:
The amino acid sequences from the helical regions of the selected zinc fingers were sequenced as:

clone 0.4/1 RSDELTRHIRIH (SEQ ID NO:30) RSDHLSKHIRTH (SEQ ID NO:37) KKFARSQTRINHTKIH (SEQ ID NO:38)
clone 0.4/2 RSDELTRHIRIH (SEQ ID NO:30) RSDHLSEHIRTH (SEQ ID NO:39) TRNARTKHTKIH (SEQ ID NO:40)
clone 0.4/4 RSDELTRHIRIH (SEQ ID NO:30) RSDHLSNHIRTH (SEQ ID NO:41) RNDTRKTHTKIH (SEQ ID NO:42)
clone 0.4/5 RSDELTRHIRIH (SEQ ID NO:30) RSDNLSTHIRTH (SEQ ID NO:43) KKFAHSNTRKNHTKIH (SEQ ID NO:44)

Paragraph beginning on page 91, line 11 has been amended as follows:
These oligonucleotides had the sequence:
tatagtTACGTGGCGatcacagtcagtccacacgtc (SEQ ID NO:45)
tatagtTGTATGGCGatcacagtcagtccacacgtc (SEQ ID NO:46)
tatagtCGTACGGCGatcacagtcagtccacacgtc (SEQ ID NO:47)

Paragraph beginning on page 92, line 10 has been amended as follows:
Some phage isolated by either of the above methods (a or b) bound DNA in the absence of ligand but could be displaced by concentrations of distamycin A at 10 μM and actinomycin D at 1 μM. The distamycin sensitive clone was selected using the DNA target AAAAAGCGGAAAAA (SEQ ID NO:48) and its helices were sequenced as:

QSRSLIQ (SEQ ID NO:49) QRDSLSR (SEQ ID NO:50) RSDERKR (SEQ ID NO:51)

Paragraph beginning on page 92, line 17 has been amended as follows:
The actinomycin D sensitive clone was selected with the DNA target AGCTTGGCG and its helices were sequenced as:

RSDELTR (SEQ ID NO:16) RSDVLST (SEQ ID NO:52) TRSSRKK (SEQ ID NO:53)

Paragraph beginning on page 93, line 20 has been amended as follows:
A method of converting zinc finger DNA binding domains to chimaeric restriction endonucleases has been described in Kim, *et al.*, (1996) Proc. Natl. Acad. Sci. USA 93:1156-1160. In order to demonstrate the applicability of DNA ligand-modulatable zinc fingers to restriction enzymes, a fusion is made between the catalytic domain of Fok I as described by Kim *et al.* and a zinc finger of Example 1. Fusion of the zinc finger nucleic acid-binding domain to the catalytic domain of Fok I restriction enzyme results in a novel endonuclease which cleaves DNA adjacent to the DNA recognition sequence of the zinc finger (AAAAAAGGCG (SEQ ID NO:14) or AAAAAAGGCGAAAAAA (SEQ ID NO:15)).

Paragraph beginning on page 93, line 29 has been amended as follows:
The oligonucleotides AAAAAAGGCG (SEQ ID NO:14) and AAAAAAGGCGAAAAAA (SEQ ID NO:15) are synthesised and ligated to arbitrary DNA sequences. After incubation with the zinc finger restriction enzyme, the nucleic acids are analysed by gel electrophoresis. Bands indicating cleavage of the nucleic acid at a position corresponding to the location of the oligonucleotide(s) (AAAAAAGGCG (SEQ ID NO:14) / AAAAAAGGCGAAAAAA (SEQ ID NO:15)) are visible.

Paragraph beginning on page 94, line 4 has been amended as follows:
In a further experiment, the zinc finger is fused to an amino terminal copper/nickel binding motif. Under the correct redox conditions (Nagaoka, M., *et al.*, (1994) J. Am. Chem. Soc. 116:4085-4086), sequence-specific DNA cleavage is observed, only in the presence of DNA incorporating oligonucleotide AAAAAAGGCG (SEQ ID NO:14) or AAAAAAGGCGAAAAAA (SEQ ID NO:15).

Paragraph beginning on page 94, line 23 has been amended as follows:
Thus, a zinc finger which recognises the DNA sequence AAAAAAGGCG (SEQ ID NO:14) is selected by phage display as described in Example 1. By the method of the preceding examples, said zinc finger is used to construct transcription factors as described above.

Paragraph beginning on page 94, line 27 has been amended as follows:
A transient expression experiment is conducted, wherein the CAT reporter gene on the reporter plasmid is placed downstream of the sequence AAAAAAGGCG (SEQ ID NO:14). The reporter plasmid is cotransfected with a plasmid vector expressing the zinc finger-HSV fusion under the control of a constitutive promoter. No activation of CAT gene expression is observed.

Paragraph beginning on page 95, line 1 has been amended as follows:
However, when the same experiment is conducted in the presence of Distamycin A, CAT expression is observed as a result of the binding of the zinc finger transcription factor to its recognition sequence AAAAAAGGCG (SEQ ID NO:14).

Paragraph beginning on page 95, line 10 has been amended as follows:
The 434 repressor is a gene regulatory protein of phage 434. It binds to a 14bp operator site (see Koudelka *et al.*, 1987, Nature vol 326 pp 886-888). This operator site consists of five conserved bp (1-5), then four variable bp (6-9), then five more conserved bp (10-14) as shown below:

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Site:  1        5  6 7 8 9 10        14
Base:  A C A A G/T X X X X A/T T T G T (SEQ ID NO:54)
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wherein X is any base.

Paragraph beginning on page 96, line 1 has been amended as follows:
Structure of target DNA sequence library:

5′ 1 6 9 14 3′
GTCGGATCCTGTCTGAGGTGAGACAATXXXXATTGTGTCTTCCGACGTCGAATTCGCG
(SEQ ID NO:55)
wherein X is any base, and the partially randomised 434 operator is underlined.

Paragraph beginning on page 96, line 19 has been amended as follows:
Oligonucleotides are then amplified by PCR, using the following primers:

Primer A 5'-GTCGGATCCTGTCTGAGGTGAG-3' (SEQ ID NO:56)
Primer B 5'-CGCGAATTCGACGTCGGAAGAC-3' (SEQ ID NO:57)

using an amplification kit (Perkin Elmer Cetus) with the following cycling regime: 93°C 30 sec; 45°C 120 sec; 45°C to 67°C ramp 60 sec; 67°C 180 sec for 25 cycles. 1 µl of eluted oligonucleotide material is used as template.

Paragraph beginning on page 97, line 12 has been amended as follows:
The operator sequence used is
5'- A C A A T A A A T A T T G T -3' (SEQ ID NO:58)

Paragraph beginning on page 99, line 6 has been amended as follows:
Using conventional cloning techniques, the sequence 5'-AAGGAGATATAACA-3' (SEQ ID NO:59) is introduced upstream of the translational initiating ATG of both pTFIIIAZifVP16 and pTFIIIAZifVP64. This sequence incorporates a plant translational initiation context sequence to facilitate translation in plant cells (Prasher *et al.* Gene 111: 229-233 (1992); Chalfie *et al.* Science 263: 802-805 (1992)).

Paragraph beginning on page 108, line 1 has been amended as follows:
[Sequence ID 1:] TFIIIA/Zif-VP16 (SEQ ID NO:1)

Paragraph beginning on page 108, line 22 has been amended as follows:
[Sequence ID 2:] TFIIIA/Zif-VP64 (SEQ ID NO:2)

Paragraph beginning on page 109, line 6 has been amended as follows:
[Sequence ID 3:] TFIIIA/Zif binding site (SEQ ID NO:3)